
S1: 3D Printing the Parts of the OPN Fluorometer

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5 In this supplement, we describe how to 3D print the components for the OPN
Fluorometer. As with the OPN Scope,¹ OPN Colorimeter,² and OPN Spec,³ we created
each piece using the free version of DesignSpark Mechanical (RS Components, Ltd.,
Corby, Northamptonshire, UK), and readers can download a copy of this software from:
<http://www.rs-online.com/designspark/electronics/eng/page/mechanical> (make sure
10 to click on the appropriate 32B or 64B icon under the “Download DesignSpark
Mechanical” heading). We also include the CAD and STL files for each part as
Supporting Information (S2 and S3, respectively), so that readers can print or modify
these components to fit their given needs. We further list the general print dimensions
and volumes for these pieces in Table S1-1, so that readers can determine whether their
15 3D printers can accommodate these components or whether other printing
arrangements need to be made.

Table S1-1. Approximate Print Dimensions (*l x w x h*) and Volumes (Build, Support) for the Components of the OPN Fluorometer

Part	Dimensions (cm)	Volumes (cm ³)
Body	7.05 x 6.65 x 5.65	157.05; 13.10
Lid	4.55 x 5.65 x 1.30	31.50; 1.25
Tube	4.55 x 5.15 x 10.55	53.50; 1.05
Plug	2.65 x 2.65 x 1.55	5.25; 0.35

As far as the individual pieces of the OPN Fluorometer are concerned (Fig. S1-1),
similar to the OPN Colorimeter and OPN Spec,^{2, 3} we designed the body of the OPN
Fluorometer (S2A, S3A) to hold a standard glass (or other clear) cuvette (external
20 dimensions: 1.25 cm x 1.25 cm x 4.5 cm) and the corresponding lid (S2B, S3B) to fit
directly over the top. We further designed the tube for the light source (S2C, S3C) to

hold an Outlite WT03 tactical LED flashlight, whose cylindrical head has a 37-mm diameter (Fig. S1-1, left). Readers, however, can change the inner diameter of this tube (as well as its length) to hold other LED flashlights instead. We also include a plug (S2D, S3D) that fits into the side of the OPN Fluorometer body and holds a sensitive light dependent resistor (LDR) for measuring the intensity of the light passing through the cuvette at a 90° angle (Fig. S1-1, right).



Figure S1-1. The 3D-printed version of the OPN Fluorometer, which holds an Outlite WT03 tactical LED flashlight. Note that we used a small piece of white PVC board in this set-up (lower right) to prop up the handle of the flashlight and, thus, level the beam.

STL Files and Test Prints

Like other OPN instruments that we have designed,¹⁻³ we created the STL files for the OPN Fluorometer using the free version of DesignSpark Mechanical (Export Options >> 3D Print (*.STL) >> Options). Once again, when making these STL files, we set the “resolution” to “custom” with a deviation of 0.05 mm and an angle of 0.1 degrees to generate smooth curves and distinct corners in the resulting parts.¹⁻³ Of course, as explained in the Supporting Information for our other papers,¹⁻³ different 3D printers and 3D printing programs may operate with different degrees of precision. As a result, we suggest printing out a few test pieces first (e.g., a portion of the tube or part of the body) to make sure that the above settings will generate the appropriate shapes and

dimensions in each part. These test prints should also reveal whether any dimensions in the OPN Fluorometer CAD files (S2) need to be changed, so that the components fit together as expected.

45 3D Printing

As with the OPN Colorimeter and OPN Spec,^{2, 3} we printed the parts for the OPN Fluorometer using a FlashForge Creator Pro 3D Printer (FlashForge USA, Rowland Heights, CA). Once again, we used 1.75-mm diameter SainSmart Black ABS Filament as both our build and support material, MakerBot Desktop as our firmware, and
50 Simplify3D as our slicing program.^{2, 3} However, as discussed in our prior papers,¹⁻³ readers could use other 3D printers, filament, or software instead.

With respect to the cost of printing the pieces of the OPN Fluorometer, depending upon the exact components used, we have found that we can print the parts to make between 12 and 15 OPN Fluorometers using 4 spools of black ABS filament, which
55 typically cost between \$20 to \$25 each (or \$80 to \$100 total) on Amazon (at November 2016 prices, not including any taxes or shipping).

Of course, as explained in our previous papers,¹⁻³ readers who lack a 3D printer can instead upload their STL files to a website like www.makexyz.com, which will print and ship parts to you for a fee. For example, in November 2016, we uploaded the STL files
60 for each part of the OPN Fluorometer to www.3dprintingpricecheck.com, which estimated that it would cost roughly \$66.55 to print the four basic components of the OPN Fluorometer in “high resolution” ABS plastic using the MakeXYZ website (not including any taxes or shipping). However, for an exact quote, readers should upload their specific STL files to their desired 3D printing website.

65 Hazards

As explained in our OPN Colorimeter and OPN Spec papers,^{2, 3} the major hazards that we have found when using the FlashForge Creator Pro to 3D print parts is that the

printing platform can become quite hot when it is in use (roughly 98° C for ABS material). Therefore, please exercise a great deal of caution when removing any part from the printer after job has finished. Alternatively, readers can wait until the platform has cooled slightly before removing the part.

Also, as we explained in the Supporting Information for our OPN Spec paper,³ 3D printing a part can release harmful nanoparticles into the air, depending on the filament and printer used. These particles can cause several serious health problems, such as asthma attacks, strokes, and even cardiac or respiratory arrest. Therefore, please be careful when working near the 3D printer, and make sure to wear the proper protective equipment (e.g., masks or respirators) when necessary. We also recommend keeping any 3D printers in a well-ventilated space to reduce any dangers caused by these nanoparticles.³ Readers should additionally review the following [PDF on 3D Printing Safety by Carnegie Mellon University](#) for several additional 3D printing safety tips that are quite helpful to know.

The Objective Lens

To help focus the beam from the flashlight and further increase the intensity of the light passing through a sample solution, the 3D-printed body of the OPN Fluorometer contains a small hole in the front face, which can hold an objective lens from a standard compound microscope (see Fig. S1-9 below). Typically, we use a 4x objective lens for this purpose, and two models that we have placed in our OPN Fluorometers are (i) a 4x objective lens from a used Carlsan microscope that we purchased on [eBay](#) for approximately \$15 (Fig. S1-2, left); and (ii) a new 4x objective lens made by AM Scope (SKU No. A4X-V300; Fig. S12, right), which we purchased on the [AM Scope website](#) for roughly \$13 (not including any taxes or shipping). As currently designed, the hole in the body of the OPN Fluorometer should fit this AM Scope lens snugly (although readers can wrap one layer of electrical tape around the objective to hold it firmly in place).

Alternatively, readers could use the “Pull” function in the free version of DesignSpark

95 Mechanical to change any of the key dimensions if need be.



Figure S1-2. Microscope objective lenses (4x) that we use with our OPN Fluorometers. A used 4x objective lens from a Carlsan microscope that we purchased on eBay (left). A new 4x objective lens made by AM Scope (SKU No. A4X-V300; right).

100 Of course, readers could use other objective lenses with the OPN Fluorometer instead (including lenses salvaged from any old or broken microscopes that readers may have on hand or find online). However, if using a different objective lens than the AM Scope lens described above, please note that the dimensions for the hole which holds this piece may need to be adjusted as well. Again, to make such a change, simply right-
105 click on the appropriate surface in the free version of DesignSpark Mechanical and then use the “Pull” function to re-size the hole to its appropriate diameter or depth.

The Light Source

After trying several different light sources, we found that the Outlite WT03 tactical LED flashlight (Fig. S1-3, far left) worked quite well in the 3D-printed version of the
110 OPN Fluorometer. This particular flashlight has three brightness settings, a convex lens, and an adjustable head for focusing the beam – which, when combined, can

deliver an intensity of up to 2,000 lumens from the light source. This high intensity beam makes the Outlite WT03 particularly well suited for fluorescence experiments. However, as explained in the Supporting Information for our OPN Scope paper,¹ we have found that, when this flashlight is left on for long periods of time, its head and handle can become rather warm. As a result, readers should carefully monitor this light if it is used in any in-class demonstrations or teaching labs.

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Figure S1-3. LED flashlights that we use with the OPN Fluorometer. From left to right, the Outlite WT03, Outlite A100, UltraFire, SkyWolfEye, and GeakLight models.

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Of course, other LED flashlights with similar features could be used with the OPN Fluorometer as well, and we have found that the Outlite A100, UltraFire, SkyWolfEye, and GeakLight models (Fig. S1-3) all work well in the instrument. Although not all of these lights are capable of generating up to 2,000 lumens of light, they are able to produce similar trends and results (Fig. S1-4) in the relative fluorescence assays that we have conducted.

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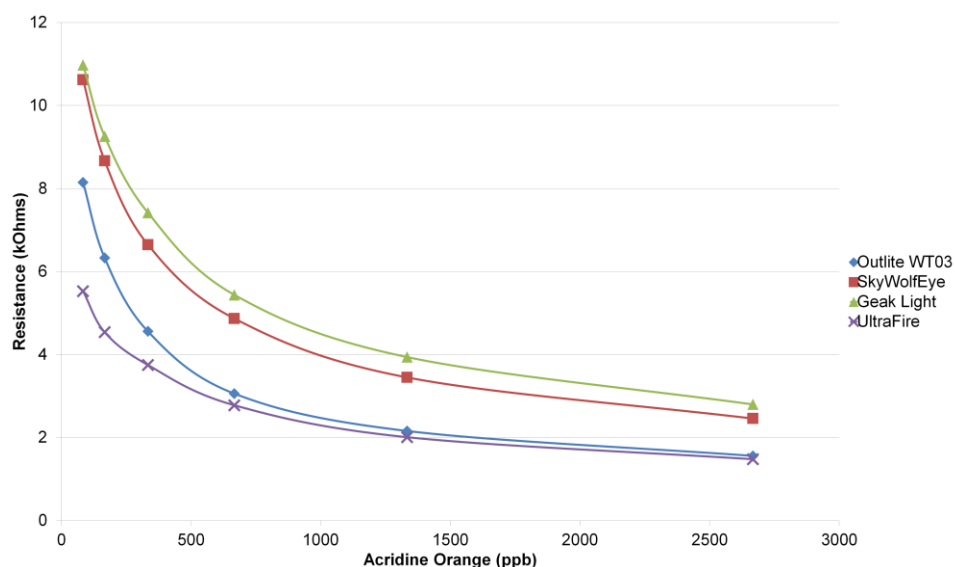


Figure S1-4. Sample concentration curves for Acridine Orange generated using different LED flashlights in the 3D-printed version of the OPN Fluorometer.

130 Nevertheless, given its larger ellipsoidal head, the Outlite A100 flashlight will not fit all of the way into the tube for the light source as it is currently designed (S2C, S3C). However, readers can insert the front portion of this flashlight into the light tube and then prop up the handle using a small block of wood or piece of PVD board to stabilize the flashlight (and, thus, the light beam). Alternatively, readers can design a larger
 135 tube to hold the Outlite A100 flashlight, but this approach may also require changing some of the dimensions of the body for the OPN Fluorometer as well.

In addition, because the other tactical LED flashlights mentioned above (i.e., the UltraFire, SkyWolfEye, and GeakLight) have smaller cylindrical heads than the Outlite WT03, we have designed separate tubes to hold each one of these three flashlights
 140 (S2E01 - S2E03, S3E01 - S3E03). Although these three tubes have the same general print dimensions as the standard tube for an Outlite WT03 flashlight (S2C, S3C), each one contains a larger volume of build material given their smaller inner diameters. As a result, according to www.3dprintingpricecheck.com, printing these tubes out on a website like www.makexyz.com would add between roughly \$9.30 and \$14.15 to the

145 cost of an OPN Fluorometer (at November 2016 prices, not including any taxes or shipping).

Alternatively, if readers have already printed out the tube to hold the Outlite WT03, we have designed small cylindrical adapters, which will fit into the larger tube to hold each one of these smaller flashlights (S2F01 - S2F03, S3F01 - S3F03; Fig. S1-5). For the convenience of readers, we include the general print dimensions and volumes for
150 these three adapters in Table S1-2 below.



155 **Figure S1-5.** Adapters for three small tactical LED flashlights that we use with the OPN Fluorometer. From left to right, the UltraFire, SkyWolfEye, and GeakLight flashlights. Readers can place these adapters into the tube for an Outlite WT03 flashlight, which should cut down on 3D printing costs.

In addition, according to the website www.3dprintingpricecheck.com, printing out any one of these three adapters on the [MakeXYZ website](http://www.makexyz.com) would cost roughly \$6.50 (at November 2016 prices, not including any taxes and shipping). Because this price is less than the cost difference for printing out the custom tubes for these flashlights

160 (S2E01 – S2E03, S3E01 – S3E03), readers may find that using the adapters is the more economical approach.

Table S1-2. Approximate Print Dimensions (*l x w x h*) and Volumes (Build, Support) for Different Flashlight Adapters for the OPN Fluorometer

Part	Dimensions (cm)	Volumes (cm ³)
UltraFire Adapter	3.75 x 3.75 x 3.05	10.05; 0.55
SkyWolfEye Adapter	3.75 x 3.75 x 3.05	14.25; 0.55
GeakLight Adapter	3.75 x 3.75 x 3.05	15.50; 0.55

Of course, to create a tube that will fit other LED flashlights, readers only need to use the “Pull” function in the free version of DesignSpark Mechanical to change the inner diameter (and possibly length) of the standard OPN Fluorometer light tube (S2C),
165 so that it can accommodate different models.

The Light Dependent Resistor (LDR) and Digital Multimeter

As our detector, we use a sensitive light dependent resistor (LDR) that is available on www.digikey.com (Fig. S1-6A, showing a NSL-6910 photocell by Luna Optoelectronics, which has a 67-kOhm “dark” resistance). Compared to the 85-cent
170 LDR that we use in the OPN Colorimeter and OPN Spec,^{2, 3} the photocell that we use in the OPN Fluorometer is more expensive, costing between roughly \$7 and \$9 each (at November 2016 prices, not including taxes or shipping). Nevertheless, we chose this specific LDR because it was one of the more sensitive ones that we found during our tests. Also, as with the OPN Colorimeter and OPN Spec,^{1, 3} we connect this LDR to a
175 digital multimeter using “jumper wires” with “alligator” clips on them, so that we can take our readings.^{4, 5} We also use the same multimeters with the OPN Fluorometer that we have used with the OPN Colorimeter and OPN Spec (Fig. S1-6B),^{2, 3} and readers should generally be able to find these or other similar models online or at a regional or national hardware chain for between \$5 and \$25 each (depending upon the exact
180 device).

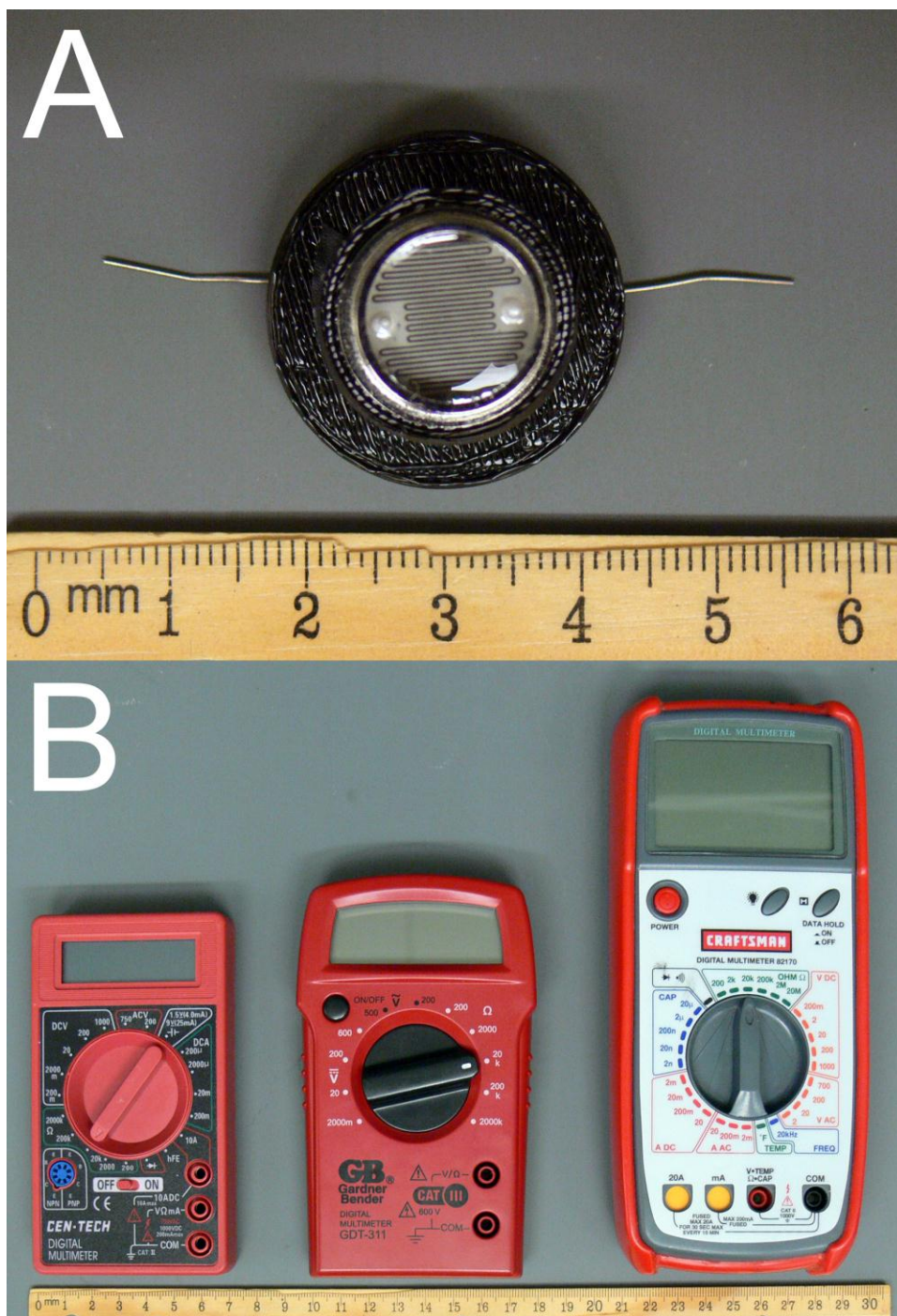


Figure S1-6. The Light Dependent Resistor (LDR) and multimeters that we use with our OPN Fluorometers. (A) NSL-6910 LDR (or photocell) by Luna Optoelectronics mounted in the 3D-printed plug for the OPN Fluorometer. (B) Cen-Tech, Gardner Bender, and Craftsman digital multimeters (from left to right).

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Of course, readers can use other LDRs or multimeters instead. However, as with the OPN Colorimeter and OPN Spec,^{2, 3} we suggest that instructors conduct an adequate number of pilot tests with these different components in the OPN Fluorometer to confirm that the set-up is working properly and generating expected results.

190 **The Cellophane Filter**

To make the excitation and emission filters for the 3D-printed version of the OPN Fluorometer, we follow a similar approach described in our OPN Colorimeter paper.² Specifically, we cut out small (roughly, 1.5-cm x 3.5-cm) pieces from different sheets or rolls of colored cellophane (Fig. S1-7). We then place the excitation filter into the “front” slot in the OPN Fluorometer (near the hole for the light tube), and we place the emission filter into the “side” slot (near the hole for the plug with the LDR). As with the cellophane filters used in the OPN Colorimeter,² these filters are not specific for a particular wavelength (e.g., 550 nm exactly). Nevertheless, we have found that students can still use these pieces of cellophane to generate useful concentration curves or other graphs for educational or instructional purposes.

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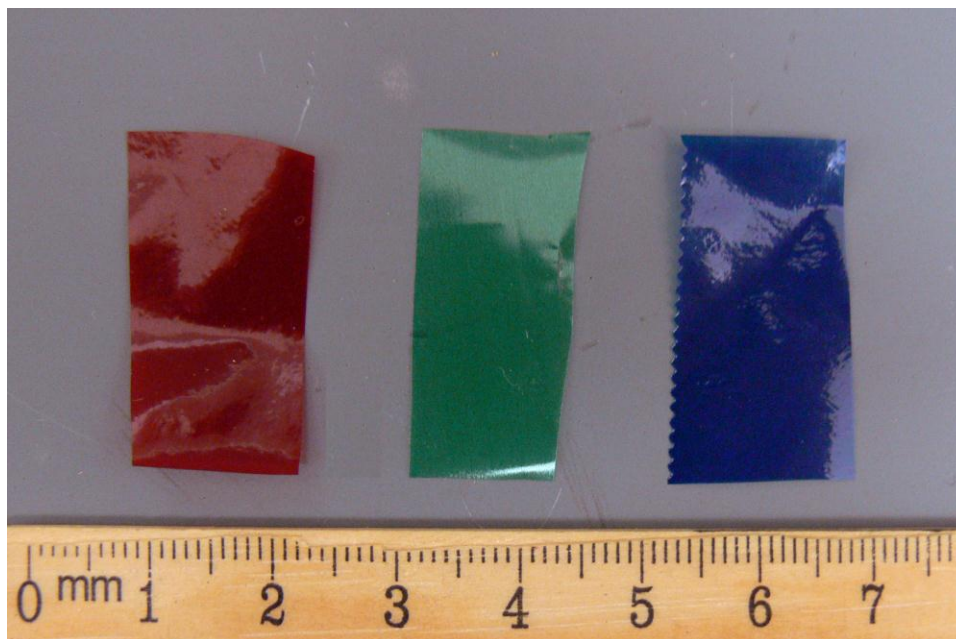
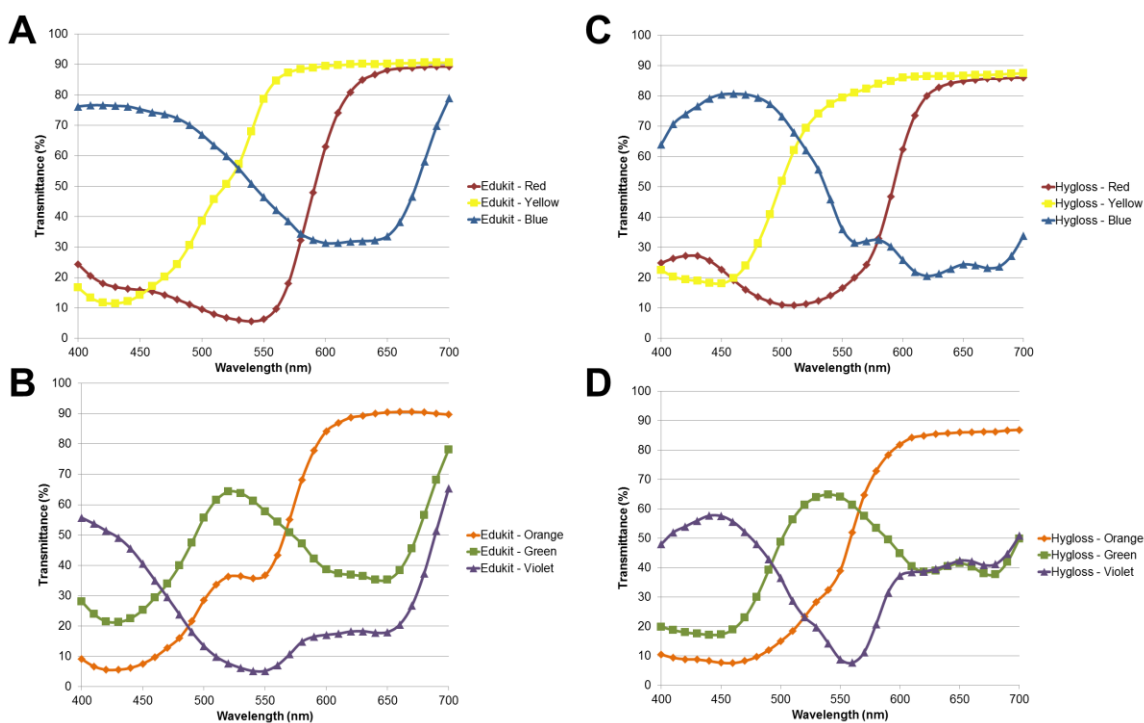


Figure S1-7. Making colored cellophane filters for the 3D-printed version of the OPN Fluorometer.

In addition, to help readers with designing or conducting their own lab activities or exercises for students, we include below (Fig. S1-8, Table S1-3) the transmission
205 spectra for two common packets of colored cellophane sheets (specifically, the Edukit and Hygloss brands) that are available on [Amazon](#) for between roughly \$6 and \$10 per set (at November 2016 prices, not including any taxes or shipping). This data is the same information contained in the Supporting Information for our OPN Colorimeter paper,² and the same is true for the related figure and table. However, we provide the
210 information again here (and summarize the steps that we took to obtain these results) for the convenience of readers.

In particular, we generated the transmission spectra for different colors of Edukit and Hygloss cellophane sheets using a commercial spectrophotometer (specially, a Hitachi U-1100), which we placed on the “Visible” setting.² We began by cutting small
215 rectangular pieces (roughly 2 cm x 5 cm) from each sheet (Red, Orange, Yellow, Green, Blue, and Violet).² Then, for each color, we taped the corresponding piece of cellophane to the far side of an empty cuvette chamber in our spectrophotometer.²

Next, we recorded transmittance values for each piece of cellophane between 400 and 700 nm in 10-nm increments.² Specifically, we first “zeroed” the
220 spectrophotometer using an empty chamber, then slid into place the test chamber with the colored cellophane, next recorded the corresponding transmittance value, and finally repeated this process for the next wavelength.² After collecting all of our data, we then graphed our results using Microsoft Excel (Fig. S1-8).²



225 **Figure S1-8.** Transmission spectra for different colors of Edukit (A and B) and Hygloss (C and D) cellophane sheets, which can be used as excitation and emission filters in the OPN Fluorometer.

Next, we examined these results to identify ranges of “useful” wavelengths for each brand of colored cellophane (Table S1-3). Specifically, we focused on ranges with transmittance values of over 45% in the violet spectrum and over 60% for the blue through red spectra.² As explained in the Supporting Information for our OPN
 230 Colorimeter paper,² although these ranges are broader than those described in a prior published report,⁶ their general values and trends are similar.

Table S1-3. Useful Wavelength Ranges for Edukit and Hygloss Colored Cellophane Sheets

Color	Edukit Range (nm)	Hygloss Range (nm)
Violet	400 to 440	400 to 480
Blue	400 to 510	400 to 520
Green	510 to 540	520 to 560
Yellow	540 to 700	510 to 700
Orange	580 to 700	570 to 700
Red	600 to 700	600 to 700

Assembling the OPN Fluorometer

After the components of the OPN Fluorometer have been 3D printed, assembling the instrument is rather easy. First, we place a glass cuvette into the body of the OPN Fluorometer and then slide their excitation and emission filters into place (in the front and side slots, respectively; Fig. S1-9A, middle). Next, we thread the leads of the LDR through the holes in the 3D-printed plug and slide the plug into place (in the side of the body; Fig. S1-9A, right). After that, we insert the objective lens into the small hole contained in the front of the OPN Fluorometer (Fig. S1-9A, middle). Then, we insert the tube for the light source into the larger hole in the front face and cover the glass cuvette with the lid for the OPN Fluorometer (Fig. S1-9B). Finally, we place the LED flashlight into the light tube (Fig. S1-9) and connect the leads of an LDR to a digital multimeter (Fig. S1-11). At the point, the OPN Fluorometer is ready to use.

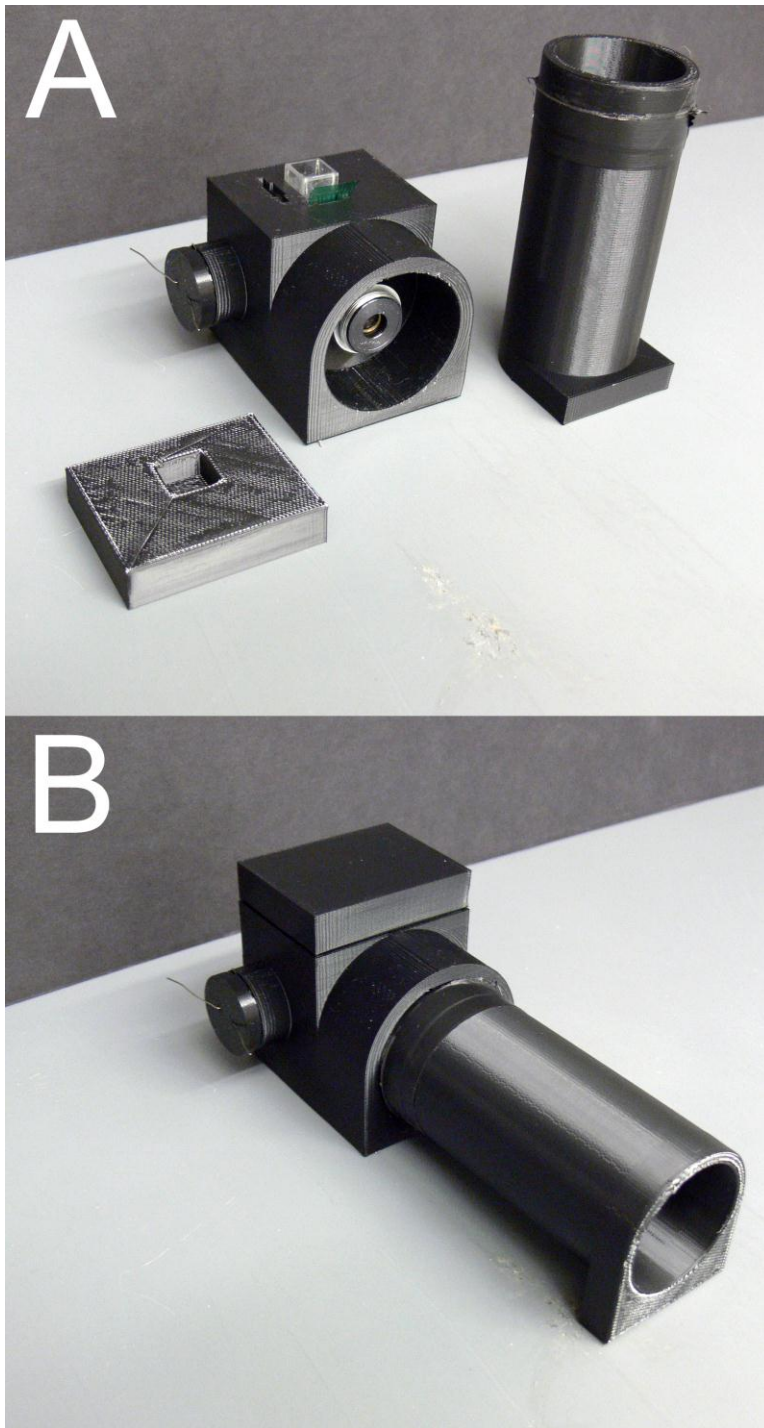


Figure S1-9. Assembling the 3D-printed version of the OPN Fluorometer from its individual parts.

A Cap and Lid to Hold a Small Test Tube

Like the OPN Colorimeter and the OPN Spec,^{2, 3} the 3D-printed version of the OPN
250 Fluorometer can also hold a small Pyrex test tube that has a 13-mm diameter (namely,
a VWR Disposable Culture Tube, No. 47729-572, which is approximately 100 mm tall).
We have therefore designed a special lid (S2G, S3G) and cover (S2H, S3H) for the OPN
Fluorometer that fits over these types of test tubes and can hold them in place (Fig. S1-
10). While we designed these two components as separate pieces in order to provide
255 easier access to the test tube when using the OPN Fluorometer, readers can merge
these parts into one or design their own (custom) piece if need be.

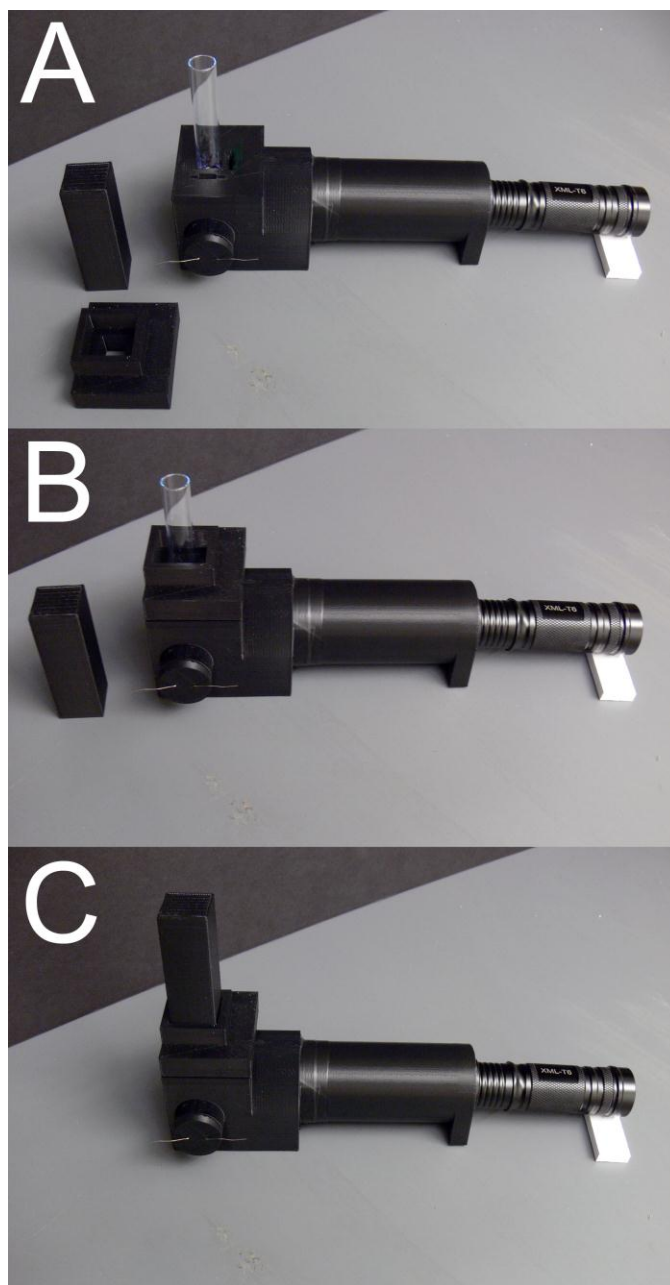
We also list the dimensions for these two parts in Table S1-4 below, and the website
www.3dprintingpricecheck.com estimates that printing these two pieces (instead the
standard lid for the OPN Fluorometer) would cost an additional \$12.35 if using [the](#)
260 [MakeXYZ website](#) (at November 2016 prices, not including any taxes or shipping).
Consequently, these components should not substantially increase the price of the OPN
Fluorometer for those schools that do not already have glass or quartz cuvettes on hand
(especially since these cuvettes can prove rather expensive – e.g., from \$10 to over \$250
each online at November 2016 prices, not including any taxes or shipping).

Table S1-4. Approximate Print Dimensions (*l x w x h*) and Volumes (Build, Support) for an OPN Fluorometer lid and cover that will fit a small test tube.

Part	Dimensions (cm)	Volumes (cm ³)
Lid – Test Tube	5.65 x 6.65 x 2.80	54.60; 5.35
Cover – Test Tube	2.35 x 2.35 x 7.05	25.10; 0.30

265 Alternatively, as long as an ultra-violet (UV) light source is not used in the OPN
Fluorometer, then students could use clear plastic (or glass) cuvettes instead of quartz
ones, and clear plastic cuvettes are much less expensive than their glass or quartz
counterparts (e.g., a box of 100 clear plastic cuvettes typically costs between \$18 and
\$40 online at November 2016 prices, not including any taxes or shipping). However,

270 please keep in mind that certain solvents (e.g., acetone and methanol) may eat away at certain plastics, so readers would likely need to use a cuvette or test tube made of glass or other similar substance for those experiments.



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Figure S1-10. A lid and cover to hold a small (13-mm x 100-mm) test tube in the 3D-printed version of the OPN Fluorometer.

Of course, readers can change the internal dimensions of the cuvette chamber in the body of the OPN Fluorometer to fit other (larger or smaller) tests tubes (simply use the “Pull” function in the free version of DesignSpark Mechanical). If so doing, however, please keep in mind that the internal dimensions of the test tube lid (S2G) and cover
280 (S2H) may also need to be adjusted to provide a better fit around the different test tube.

Helpful Hints for the OPN Spec

In light of our experiences with the 3D-printed version of the OPN Fluorometer, we provide the following helpful hints for its use. While some of these suggestions are similar to those for using the OPN Scope,¹ OPN Colorimeter,² or OPN Spec,³ we repeat
285 these hints here for the convenience of readers.

First, as with other OPN instruments that we have designed,¹⁻³ we typically use compression fittings to connect certain pieces together in our OPN Fluorometers (e.g., when we fit the light tube into the front face of the body and insert the plug into the side of the body). This is why each hole is a little larger than its corresponding tube or
290 plug. Of course, readers can change the dimensions of any part in the free version of DesignSpark Mechanical to provide a tighter fit. However, given the variations in 3D printing error tolerances discussed earlier, we prefer to wrap a few layers of electrical (or other) tape around the end of a component to achieve the same result (Figs. S1-9 and S1-10). In fact, readers can even wrap a single layer of tape around the head of an LED
295 flashlight or the cylindrical end of a 4x objective lens to hold these pieces in place more firmly as well.

Second, as explained in our OPN Colorimeter and OPN Spec papers,^{2, 3} multimeters are frequently sold with probes that cannot be attached directly to the leads of an LDR. However, in these instances, readers can simply use “jumper wires” with “alligator clips”
300 on them to connect the leads of the LDR to those of the probe (Fig. S1-11).

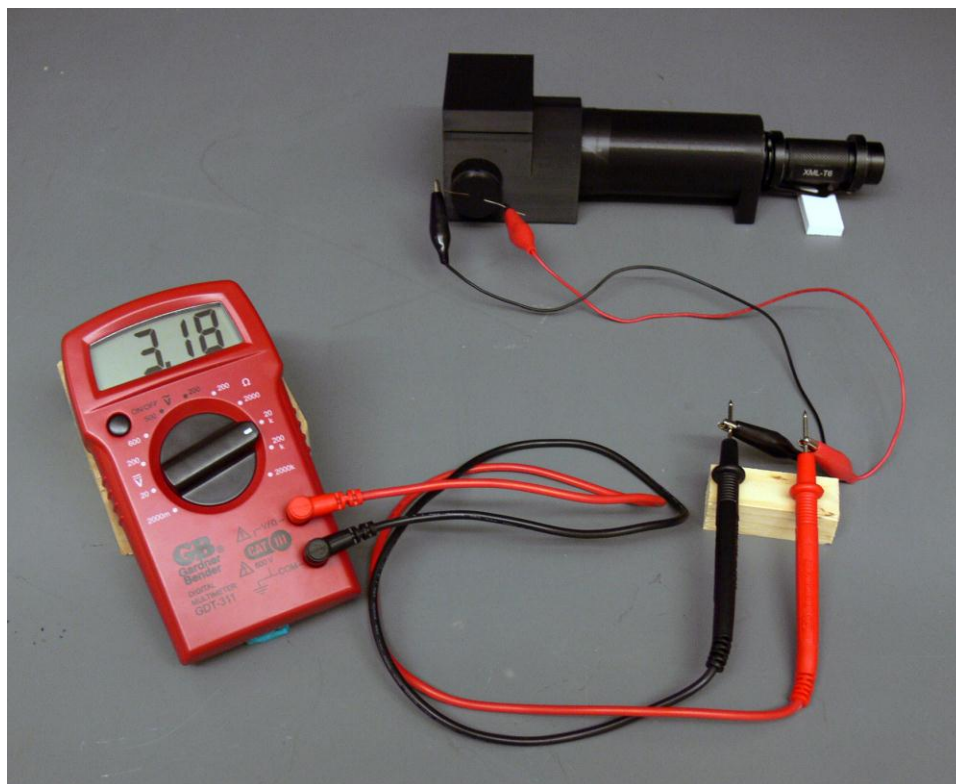
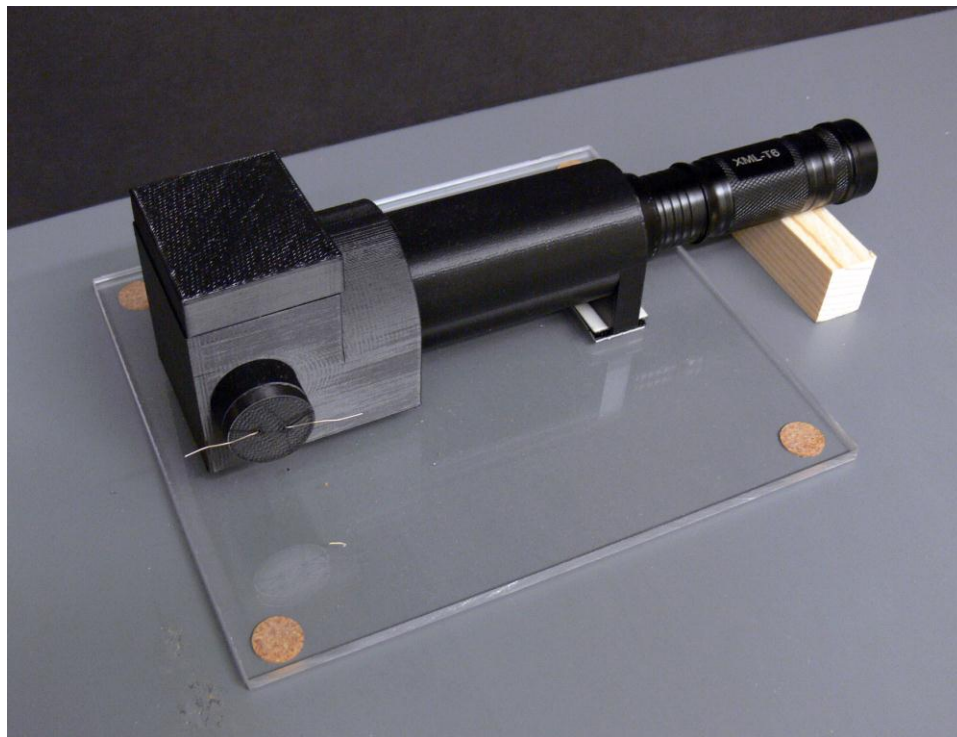


Figure S1-11. Connecting the probes of a digital multimeter to the leads of an NSL-6910 light dependent resistor (LDR) in the OPN Fluorometer using “jumper wires” with “alligator clips” on them.

Third, like the OPN Colorimeter and OPN Spec,^{2, 3} we usually hold our OPN
305 Fluorometers in place on the table top using small pieces of removable poster tack or
mounting putty. We take this approach because, once students have begun an
experiment, it is very important that their OPN Fluorometers do not accidentally move
around (e.g., as a result of being bumped or nudged) since these changes can affect the
amount or intensity of the light striking the cuvette, which might render subsequent
310 readings incomparable to earlier ones.^{2, 3}

Fourth, alternatively, as discussed in our OPN Colorimeter and OPN Spec papers,^{2, 3}
instructors may instead want to use Velcro strips, Scotch “Extreme” Fasteners®, or
other similar connectors to secure the parts of an OPN Fluorometer onto a thin sheet of
plastic (Fig. S1-12). This approach might make it easier for students to set up, use, and
315 then put away the instrument. Plus, given the limited number of pieces involved and

the way in which they are put together, readers will not need to conduct any initial pilot tests to make sure that the parts are arranged properly (unlike with the OPN Spec).³



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Figure S1-12. Securing the OPN Fluorometer to a thin sheet of clear plastic using Scotch “Extreme” Fasteners ®. Readers can alternatively use Velcro strips or other adhesive connectors. Also, note how we use a small wooden block to prop up the handle of the flashlight, which helps to stabilize the beam.

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Fifth, for these same reasons, instructors may want their students to tape their flashlights into place as soon as they have properly placed them in their OPN Fluorometers. In the process, students should make sure that the initial position of the flashlight generates a reading on the multimeter that will lead to useful results as their experiments progress.

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Sixth, for this reason, we recommend that instructors conduct some “test runs” of their planned lab exercises or activities in advance to have a better understanding of the resistance values that students should be getting during the lab. These test runs should also help instructors “trouble shoot” a lab, enabling them to better anticipate (and resolve) any issues that their students may encounter during the activity. Plus, if

using a different LDR or flashlight than the ones described above (or a different fluorophore than the ones described in the Supporting Information, S5), then a set of
335 test runs would be particularly appropriate.

Seventh, to reduce the amount of ambient light that could strike the cuvette during an assay, which might lead to fluorescent bleaching, instructors may want to consider dimming (or turning off) the overhead lights – as long as this would not create any safety hazards during the activity. Alternatively, instructors could light the room with
340 one or more clear 25-W bulbs (or similar light sources), which should provide sufficient light for students while they conduct their experiments.

Eighth, as with the OPN Colorimeter and OPN Spec,^{2,3} we recommend turning on the LED flashlight and placing it in the OPN Fluorometer light tube at least 10 minutes before beginning any experiment in order to give these components a sufficient time to
345 warm up (S5). Furthermore, at the start of this warm-up period, we suggest that students place a clear cuvette or test tube filled with their solvent in the OPN Fluorometer and cover it with the appropriate lid to obtain a “blank” reading (S5). Then, as with the OPN Colorimeter and OPN Spec,^{2,3} students should record the resistance value displayed on their multimeters at fixed (e.g., 1-minute) intervals in
350 order to evaluate how quickly the set-up is stabilizing (e.g., in our experience, a rate of change that is less than 1% per minute appears to indicate a fairly stable set-up).

Finally, readers should know that, like the PDV-P8103 LDR that we use with the OPN Colorimeter and OPN Spec,^{2,3} the resistance of the NSL-6910 photocell is inversely proportional to the intensity of the light striking it. As a result, the graphs generated
355 with this LDR will appear to be “flipped” versions of a typical calibration curve, which decrease (instead of increase) with concentration levels before eventually leveling off (S5). Thus, to avoid any confusion among the students, instructors may want to explain this phenomenon at the start of lab (S5). Alternatively, students can subtract

the resistance values associated with each of their individual fluorophore solutions from
360 their initial “blank” reading to obtain graphs that look like more traditional calibration
curves, and we discuss this technique in greater detail in the Supporting Information
describing our protocols for conducting relative fluorescence assays with the OPN
Fluorometer (S5).

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